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Methods

Reactive Sulphydryl Groups present in Horse (Equus Ferus Caballus) Carbonmonoxyhaemoglobin by Titrating with 5, 5'-Dithiobis-2-Nitrobenzoate (DTNB)

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Abstract

This research was carried out to determine the number of reactive sulphydryl groups in horse (Equus ferus caballus) haemoglobin. Haemolysate was prepared from the horse blood. The haemoglobin was separated into major and minor fractions using carboxymethyl cellulose (CMC-52). Each haemoglobin fraction, in phosphate buffer pH 7.6, was accurately measured into several clean, dry test tubes. Increasing volumes of stock 5,5'-dithiobis-2-nitrobenzoate (DTNB) were added to the different test tubes and left to equilibrate for 3 hours. Absorbance of each solution in each test tube was read at 412 nm. A graph of change in absorbance against the volume of DTNB was plotted; maximum change in absorbance was obtained at the point where the graph levels off. The ratios of 5-thio-2-nitrobenzoate (TNB) concentration to the concentration of haemoglobin tetramer (Hb4) were calculated and plotted against the volume of DTNB. The number of sulphydryl groups reacting with DTNB was found to be two in both the major and minor haemoglobin.

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